BIOFERTILIZERS WITH NATURAL PHOSPHATE, SULPHUR AND *Acidithiobacillus* IN A SOIL WITH LOW AVAILABLE-P

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ABSTRACT: The production of mineral fertilizers is a expensive process, since it requires high energy consumption, and cannot be produced by small farmers. Laboratory assays were conducted to produce P-biofertilizers from natural phosphate (B_5, B_{10}, B_{15}, B_{20}), applying sulphur at different rates (5; 10; 15 and 20%) inoculated with *Acidithiobacillus* (S*) and testing increasing periods of incubation. A greenhouse experiment was carried out to evaluate the effect of the biofertilizers in a soil with low available P (Typic Fragiudult) from the "Zona da Mata" of Pernambuco State, grown with yam bean (*Pachyrhizus erosus*) in two consecutive harvests. The treatments were: Natural Phosphate (NP); biofertilizers produced in laboratory (B_5, B_{10}, B_{15}, B_{20}) with sulphur and *Acidithiobacillus* (NP+S*); natural phosphate with sulphur (20%) without *Acidithiobacillus* (NP+S); triple super phosphate (TSP) and a control without phosphorus. Plants were inoculated with a mixture of rhizobia strains (NFB 747 and NFB 748) or did not receive rhizobia inoculation. In bioassays pH and available P in the biofertilizers were analyzed. In the greenhouse experiment shoot dry matter, total N and total P in shoots, soil pH and available P were determined. Higher rates of available P were obtained in biofertilizers with sulphur and *Acidithiobacillus* (NP+S*) and in triple super phosphate (TSP), and biofertilizers with sulphur and *Acidithiobacillus* (FN+S*) and triple super phosphate (TSP) increased plant parameters. Native rhizobia were as effective as the strains applied in inoculation. After the two harvests soil presented lower pH values and higher rates of available P when the biofertilizers B_{15} and B_{20} with sulphur and *Acidithiobacillus* were applied.
Key words*: Pachyrhizus erosus,* P uptake, phosphorus fertilization, yam bean

BIOFERTILIZANTES COM FOSFATO NATURAL, ENXOFRE E *Acidithiobacillus* EM SOLO COM BAIXO P DISPONÍVEL

RESUMO: A produção de biofertilizantes é um processo prático e econômico que reduz o consumo de energia e a sua aplicação visa aumentar o fósforo (P) disponível no solo. Biofertilizantes fosfatados (B_5, B_{10}, B_{15}, B_{20}) produzidos em laboratório usando fosfato natural (FN) e enxofre em diferentes concentrações (5; 10; 15 e 20%), inoculado com *Acidithiobacillus* (S*) e enxofre (20%) sem *Acidithiobacillus* (S), com incubação por 30, 45 e 60 dias, foi utilizado em um experimento em vasos com solo da Zona da Mata de Pernambuco (Argissolo Amarelo), com baixo nível de P disponível, cultivado com jacatupé (*Pachyrhizus erosus*), por dois períodos consecutivos. Os tratamentos fosfatados foram: fosfato natural (FN); biofertilizantes (B_5, B_{10}, B_{15}, B_{20}), com *Acidithiobacillus* (FN+S*) e $B_{20,}$ sem *Acidithiobacillus* (FN+S); superfosfato triplo (ST); e um controle sem fósforo (P_0). As plantas foram inoculadas com a mistura das estirpes de rizóbio NFB 747 e 748, e sem inoculação. Nos ensaios em laboratório analisou-se o pH e o P extraído dos biofertilizantes, em cada período de incubação, e nos experimentos em vasos com solo determinou-se a biomassa seca, o N e o P total acumulado da parte aérea, o pH e o P extraído do solo. O P disponível foi mais elevado para os biofertilizantes com *Acidithiobacillus* (FN+S*) e para o superfosfato triplo. A fertilização fosfatada, condicionou resposta positiva dos biofertilizantes (FN+S*) e do superfosfato triplo. Os rizóbios nativos foram tão eficientes quanto os do inoculante. Após os dois cultivos o P disponível foi maior com os biofertilizantes B_{15} e B_{20} com *Acidithiobacillus*.
Palavras-chave: Pachyrhizus erosus, jacatupé, fertilização fosfatada, solo ácido

INTRODUCTION

Yam bean (*Pachyrhizus erosus* L. Urban) is a legume that produce high yields of tubers (60 t ha^{-1}) and grains (4 t ha^{-1}), with high percentage of edible oil and protein similar to soybean (Sorensen, 1996). The botanical and genetic characteristics of yam bean have been studied by the International Yam bean Program in Denmark and researches with rhizobia effectiveness and response to fertilizers are realized in Brazil by the Nucleus of Biologic Nitrogen Fixation in the Tropics (NFBNT) at the Federal Rural University of Pernambuco.

Strains of rhizobia previously screened for yam bean submitted to higher temperature conditions (Stamford et al., 1995; 1999) were very effective, and in some Brazilian soils, nitrogen fixation by these selected strains may be sufficient to supply nitrogen for good yields (Cruz et al., 1997). To optimize the biological nitrogen fixation may be necessary nutrient application, especially phosphorus, because of the numerous functions exercised by this element in the symbiotic process (Burity et al., 2000).

The basic materials for production of phosphorus fertilizers is phosphoric rocks. The most commonly used is apatite a no-restorable resource. The production of P-soluble fertilizers, such as super phosphates and thermo phosphates, requires higher energy consumption, specific strategies, and conduction of researches for the establishment of efficient and economic use of natural phosphates (Goedert & Sousa, 1986). The immediate utilization of phosphoric rock in the natural form is very restricted because of the low solubility, turning a more common practice perennial crops, mixed with soluble fertilizers, maintaining a slow nutrient release and uptake (Oliveira et al., 1977).

Studies on the isolation and selection of microorganisms with ability to promote higher solubilization of phosphoric rocks have been carried out in many works, especially because of the possibility of interaction with microorganisms involved in biological nitrogen fixation (Nahas, 1999). The bacteria involved in the process of biological nitrogen fixation may interact with microorganisms that realize phosphoric rock solubilization, especially with the bacteria from the genus *Acidithiobacillus*, which react with sulphur producing sulphuric acid (Garcia Jr., 1992) and promote intense phosphoric rock solubilization with higher availability of phosphorus in soil, and that, in turn, is favorable to the symbiotic process and plant growth (Santos, 2002).

It is necessary to evaluate and compare the effects of the application of sulphur inoculated with *Acidithiobacillus* in plant growth and in soil reaction to P soluble fertilizers and natural phosphate, because the sulphuric acid produced in the biological reaction could act in the natural phosphate solubilization and in the soil reaction reducing soil pH, and that could hamper plant growth (Stamford et al., 2002).

This study was carried out to evaluate the effect of biofertilizers produced from natural phosphate (Gafsa) and sulphur inoculated with *Acidithiobacillus* in a soil with low level of available phosphorus, comparing to P soluble fertilizer (triple super phosphate) and natural phosphate on nodules and shoot biomass, total N and total P accumulation on shoot, soil pH and available P. The effect of rhizobia inoculation with strains selected for effective N_2 fixation on yam bean and the interaction between inoculation with rhizobia strains and phosphorus treatments was also evaluated.

MATERIAL AND METHODS

Assays using plate dishes and plastic trays (50 cm x 30 cm x 5 cm) for biofertilizers production using natural phosphate (Gafsa) with addition of sulphur at different rates (5, 10, 15 and 20%) inoculated with *Acidithiobacillus* (S*) were conducted in laboratory. Bacterial cultures were grown in 125 Erlenmeyer flasks using medium 9K (Garcia Jr, 1991) for 5 days at 180 rpm in a horizontal shaker at 28-30°C. Inoculation was applied at a rate of 1 mL g^{-1} of sulphur. Biofertilizer with natural phosphate and sulphur (20%) without *Acidithiobacillus* inoculation (S) was also produced. During the incubation period (30, 45 and 60 days), boiled, filtered water was added to keep samples at 80% of saturation, monitored by daily weighing.

The biofertilizers were produced in trays using natural phosphate (2 kg) plus elemental sulphur at the same rates (5; 10; 15 and 20%) as for plate dishes, and incubated for 60 days. At the end of the incubation periods pH and available P (Mehlich 1) were determined in Petri dishes and in trays, according to EMBRAPA (1997) methodology. The biofertilizers produced in trays were applied in the greenhouse experiment carried out in pots (8 dm^3), grown with yam bean legume (*Pachyrhizus erosus* (L) Urban). Plants were harvested in two consecutive crops after 90 days of planting.

A Typic Fragiudult with low available P was used. The soil was collected in the District of Carpina, Zona da Mata region located in Pernambuco State, Northeast of Brazil. Soil samples (0-30 cm layer) were sieved (5 mm), mixed and placed in plastic pots. Results from soil analyses (eight replications) are pH (H_2O 1:2.5) 5.9; exchangeable cations ($mmol_c$ kg^{-1}) Ca^{2+} 14.0, Mg^{2+} 12.8, K 1.7; P (Mehlich 1) 2.7 mg kg^{-1}; total N 0.6 g kg^{-1}; Organic C 8.2 g kg^{-1}; dp 2.66 kg dm^{-3}; dg 1.45 kg dm^{-3}; sand, silt and clay contents 760; 100 and 140 g kg^{-1}, respectively.

The pot experiment was arranged in a completely randomized factorial design with three replications (Silva & Silva, 1982). P treatments were: natural phosphate (NP) in the commercial status; biofertilizers produced in laboratory (B_5, B_{10}, B_{15}, and B_{20}) using natural phosphate with elemental sulphur in the rates 5, 10, 15 and 20% inoculated with *Acidithiobacillus* (NP+S*); natural phosphate with sulphur 20% without *Acidithiobacillus* (NP+S); triple super phosphate (TSP); and control no P fertilization (P_0). P fertilization was applied following the maximum recommendation for yam bean according to Stamford et al. (1999), equivalent to 100 kg P ha^{-1}. Fertilizers were applied 10 cm below and 10 cm side way, based on results obtained by Stamford et al. (1990).

Rhizobia treatments were performed with or without inoculation. Seeds of yam bean were inoculated with strains NFB 747 and NFB 748, selected for efficient nitrogen fixation on yam bean in previous experiments carried out in acid and high temperature conditions (Stamford et al., 1995; 1999). Inoculant was prepared in YM medium using 125 mL Erlenmeyers maintained in a horizontal shaker at 100 gpm, for 5 days, at 28-30°C (containing greater than 10^8 g^{-1} in liquid culture). A mixture of both strains was used for inoculation (1:1) and applied 1 mL per plant. In the consecutive growth plants were reinoculated with rhizobia following the same process described for the first crop.

Lime was not applied because of the adequate values of soil pH (5.9) and exchangeable cations (28 $mmol_c$ kg^{-1}) for tropical legumes. To reduce the influence of humidity changes on available P in soil, as reported by Ruiz et al. (1990), water was applied twice a day (8h00 and 17h00), to keep moisture at 80% of field capacity. In the materials used as P sources, before application of the P treatments, available P (Mehlich 1) following the EMBRAPA (1997) methodology was analyzed.

In the first experiment shoots were harvested 90 days after seeds emergence (DAE), and two leaves per plant were not harvested to promote new growth of yam bean plants, aiming to estimate the residual power of P fertilizers in two consecutive crops. In the later experiment, plants were harvested 90 days after the first harvest. Nodules and shoot dry matter; total N and total P in shoots; soil pH and available P (Mehlich 1) were determined. Total N was analyzed by Kjeldhal semimicro method (Kjeltec 1030), and total P in shoots determined following Malavolta et al. (1989). Soil pH (water 1.0:2.5) and available P (Mehlich 1) were determined according to EMBRAPA (1997).

The software SANEST (Zonta et al., 1982) was used for statistical analysis of data and treatments means were compared by the Tukey test (P = 0.05) (Silva & Silva, 1982).

RESULTS AND DISCUSSION

Laboratory assays

The content of available phosphorus in the assays for biofertilizers production in Petri dishes, collected in the different incubation periods showed liberation of P from biofertilizers, increasing three times the results of natural phosphate, and no differences were observed between the periods of incubation (Table 1). The results obtained in trays incubated for 60 days were inferior to available P in assays with Petri dishes, although the biofertilizers produced from natural phosphate and sulphur inoculated with *Acidithiobacillus* showed potential in phosphorus availability (Table 1).

Biofertilizers with sulphur and *Acidithiobacillus* (NP+S*) and soluble fertilizer (triple super phosphate) did not show difference, and biofertilizer with sulphur without *Acidithiobacillus* (NP+S) are not different of natural phosphate without sulphur (FN). In general, biofertilizers with sulphur and *Acidithiobacillus* decreased pH values, and showed a clear effect of H_2SO_4 produced by the microbiological reaction carried out by *Acidithiobacillus* bacteria.

Greenhouse experiment

Shoot dry matter of yam bean in the two harvests were not different under fertilization with either natural phosphate with sulphur and *Acidithiobacillus* (NP+S*) or triple super phosphate (TSP), which showed higher dry biomass of shoots compared to the others P treatments (Table 2). Biofertilizer with sulphur without

Table 1 - Available P (Mehlich 1) and pH of biofertilizers produced in laboratory assays and triple super phosphate.

P-sources	Periods of incubation in Petri dishes			in trays	
	30 days	45 days	60 days	60 days	
	-------------------- Available P - Mehlich 1 (%)			--------------------	pH
Natural phosphate (NP)	1.9 bA	1.9 bA	2.0 bA	1.1 c	5.4 a
Biofertilizer $_{20}$ (NP+S)	-	-	-	1.6 c	5.2 a
Biofertilizer $_{5}$ (NP+S*)	6.5 aA	7.6 aA	7.7 aA	4.7 b	4.2 b
Biofertilizer $_{10}$ (NP+S*)	6.1 aA	6.5 aA	6.5 aA	5.5 ab	4.1 b
Biofertilizer $_{15}$ (NP+S*)	5.8 aB	5.8 aB	7.7 aA	6.0 a	4.1 b
Biofertilizer $_{20}$ (NP+S*)	6.1 aA	6.5 aA	7.0 aA	6.1 a	4.1 b
Triple super phosphate (TSP)	-	-	-	6.0 a	5.0 b
C.V. (%)		8.93		7.56	6.78

[1](NP+S*) = natural phosphate plus sulphur inoculated with *Acidithiobacillus*. (P+S) = natural phosphate plus sulphur without *Acidithiobacillus*.

[2]Values followed by different letters are different (P = 0.05), using the Tukey test. Upper case letters compare data in rows and lower letters in columns.

Acidithiobacillus (NP+S) yielded greater yam bean shoot dry matter than the natural phosphate (NP) and control treatment without P fertilizer (P_0).

Dry biomass of plants did not vary with and without rhizobia inoculation. Although rhizobia strains led to better results compared to uninoculated plants, in the two consecutive harvests there was no effect of rhizobia inoculation.

Phosphorus application affected total nitrogen yields in yam bean shoot dry matter in the first harvest, and in the absence of phosphorus (P_0), yam bean grew poorer and accumulated lower amount of total nitrogen, although natural phosphate (NP) and biofertilizer with sulphur without *Acidithiobacillus* (NP+S) produced no significant response to total nitrogen accumulation, in comparison to control treatment without application of phosphorus (Table 3). Overall, in treatments with rhizobia inoculation, there was not effect of P application, except when the biofertilizer B_{15} with *Acidithiobacillus* (NP+S*) was applied.

In the consecutive harvest, in general, there was effect of phosphorus application compared to the treat-

Table 2 - Effects of P treatments on in shoot dry biomass of yam bean inoculated and uninoculated with rhizobia grown in a soil with low available P in two consecutive harvests.

P-sources	Harvest 1			Harvest 2		
	Inoculated	Uninoculated	Means	Inoculated	Uninoculated	Means
	Shoot dry biomass, g planta^{-1}					
Control with no P (P_0)	2.39 cA	2.39 cA	2.39 b	2.24 bA	1.98 bA	2.11 b
Natural phosphate (NP)	2.94 bcA	2.58 bcA	2.76 b	3.58 abA	2.34 abB	2.96 b
Biofertilizer $_{20}$ (NP+S)	3.56 aA	3.17 aA	3.37 a	3.44 abA	3.18 aA	3.31 ab
Biofertilizer $_{5}$ (NP+S*)	2.69 cA	2.62 bcA	2.66 b	2.99 bA	2.93 abA	2.96 b
Biofertilizer $_{10}$ (NP+S*)	3.42 abA	3.33 aA	3.37 a	4.37 aA	3.51 aA	3.94 a
Biofertilizer $_{15}$ (NP+S*)	3.84 aA	3.33 aA	3.58 a	4.08 aA	3.35 aA	3.72 a
Biofertilizer $_{20}$ (NP+S*)	3.45 abA	3.16 aA	3.31 a	4.12 aA	2.79 abA	3.43 ab
Triple super phosphate	3.69 aA	3.03 abA	3.36 a	4.12 aA	3.03 aA	3.57 ab
Means	3.25 A	2.96 B		3.62 A	2.88 B	
C.V. (%)		7.47			3.44	

[1](NP+S*) = natural phosphate plus sulphur inoculated with *Acidithiobacillus*. (P+S) = natural phosphate plus sulphur without *Acidithiobacillus*.
[2]Values followed by different letters are different (*P* = 0.05), using the Tukey test. Upper case letters compare data in rows and lower letters in columns.

Table 3 - Effects of P treatments on total N in shoot dry biomass of yam bean inoculated and uninoculated with rhizobia grown in a soil with low available P in two consecutive harvests.

P-sources	Harvest 1			Harvest 2		
	Inoculated	Uninoculated	Means	Inoculated	Uninoculated	Means
	Total N in shoot dry biomass, mg planta^{-1}					
Control with no P (P_0)	48 bA	42 bA	45 b	68 bA	60 bA	64 b
Natural phosphate (NP)	72 abA	62 abA	67 ab	135 aA	80 abB	107 a
Biofertilizer $_{20}$ (NP+S)	79 abA	87 aA	83 a	118 aA	115 aA	116 a
Biofertilizer $_{5}$ (NP+S*)	66 abA	65 abA	66 ab	133 aA	79 abA	106 a
Biofertilizer $_{10}$ (NP+S*)	81 abA	83 aA	82 a	132 aA	112 aA	122 a
Biofertilizer $_{15}$ (NP+S*)	96 aA	82 aA	89 a	132 aA	104 aB	118 a
Biofertilizer $_{20}$ (NP+S*)	81 abA	88 aA	85 a	131 aA	103 aA	117 a
Triple super phosphate	84 abA	84 aA	84 a	137 aA	107 aA	122 a
Means	76 A	74 A		123 A	95 B	
C.V. (%)		16.93			16.27	

[1](NP+S*) = natural phosphate plus sulphur inoculated with *Acidithiobacillus*. (P+S) = natural phosphate plus sulphur without *Acidithiobacillus*.
[2]Values followed by different letters are different (*P* = 0.05), using the Tukey test. Upper case letters compare data in rows and lower letters in columns.

ment without phosphorus. However, plants without rhizobia inoculation showed not response to natural phosphate and biofertilizers with sulphur without *Acidithiobacillus* inoculation (NP+S) compared to control treatment (P_0). In the two consecutive harvests, the best results of total nitrogen accumulation in shoots were obtained for plants inoculated with specific rhizobia strains, but the effect of rhizobia inoculation was significant only in the consecutive harvest.

Total P accumulation in dry matter shoots of yam bean produced significant response when the phosphorus sources were applied, especially with application of biofertilizers with sulphur and *Acidithiobacillus* (NP+S*) and triple super phosphate (TSP). In the first harvest, natural phosphate (NP) and biofertilizer without *Acidithiobacillus* (NP+S) were not different to control treatment without phosphorus application (Table 4). In the later harvest, biofertilizers with sulphur and without *Acidithiobacillus* (NP+S) led to greater total phosphorus accumulation, probably because of the presence of native *Acidithiobacillus* bacteria which, in the second harvest, produced sulphuric acid sufficient to promote phosphorus solubilization of natural phosphate.

In the two consecutive harvests it was not observed response to rhizobia inoculation in total phosphorus in shoot dry matter of yam bean, although in the later harvest, significant response of rhizobia inoculation was observed when biofertilizers (B_{15} and B_{20}) with sulphur and *Acidithiobacillus* (NP+S*), B_{20} with sulphur without *Acidithiobacillus* (NP+S) and natural phosphate (NP) were applied.

Results of soil pH and available P (Mehlich 1), determined at the end of the later harvest, are presented in (Table 5). Soil pH decreased by application of biofertilizers with sulphur and *Acidithiobacillus* (FN+S*), especially when applied biofertilizers with higher levels of sulphur, indicating the effect of sulphuric acid formed during sulphur oxidation promoted by *Acidithiobacillus*. Application of B_{20} biofertilizer with sulphur and without *Acidithiobacillus* decreases soil pH. This effect probably resulted from sulphuric acid produced by native *Acidithiobacillus* which during the long experimental period (180 days) could produce sufficient sulphur oxidation.

In the biofertilizers, the sulphur bacteria *Acidithiobacillus* elicits the reaction of sulphur with water and oxygen, forming higher amounts of sulphuric acid (Garcia Jr., 1992) at varying the rates, as related to the different amounts of elemental sulphur applied. The sulphuric acid produced reacted with the natural phosphate increasing the available P and lowered pH, according to the amount of sulphur in the different biofertilizers and depending on the period of incubation, with consistent results.

The effects of the P treatments on shoot biomass compared with applying natural phosphate (NP) in the commercial status and the control treatment without applying P are conclusive. Similar results were reported by Santos (2002), evaluating the effect of P biofertilizers and soluble fertilizer (triple super phosphate) on mimosa tree legume grown in an acid soil. Klepker & Anghinoni (1995) studying the effect of phosphorus application in maize, reported greater response of soluble fertilizers compared with natural phosphates.

Results of rhizobia inoculation were not as efficient as the results of the experiments carried out by

Table 4 - Effects of P treatments on total P in shoot dry biomass of yam bean inoculated and uninoculated with rhizobia grown in a soil with low available P in two consecutive harvests.

P-sources	Harvest 1			Harvest 2		
	Inoculated	Uninoculated	Means	Inoculated	Uninoculated	Means
	------------------------ Total P in shoot dry biomass, mg planta^{-1} -------------------------					
Control with no P (P_0)	0.55 cA	0.60 bA	0.57 d	0.51 bA	0.57 bA	0.54 c
Natural phosphate (NP)	0.72 bcA	0.67 abA	0.69 cd	0.93 aA	0.56 bB	0.74 bc
Biofertilizer $_{20}$ (NP+S)	0.99 abA	0.91 abA	0.95 ab	0.89 abA	0.77 abA	0.83 ab
Biofertilizer $_{5}$ (NP+S*)	0.81 abcA	0.68 abA	0.75 bcd	1.20 aA	0.69 abB	0.94 ab
Biofertilizer $_{10}$ (NP+S*)	1.06 abcA	0.98 aA	0.89 abc	1.14 aA	1.02 aA	1.08 a
Biofertilizer $_{15}$ (NP+S*)	1.02 aA	0.90 abA	0.98 a	0.83 abA	0.84 abA	0.84 ab
Biofertilizer $_{20}$ (NP+S*)	1.01 abA	0.96 aA	0.99 a	1.10 aA	0.67 abB	0.88 ab
Triple super phosphate	0.99 abA	0.91 abA	0.96 ab	1.16 aA	0.66 abB	0.91 ab
Means	0.89 A	0.82 A		0.97 A	0.72 B	
C.V.(%)		14.52			17.37	

[1](NP+S*) = natural phosphate plus sulphur inoculated with *Acidithiobacillus*. (P+S) = natural phosphate plus sulphur without *Acidithiobacillus.*

[2]Values followed by different letters are different (P = 0.05), using the Tukey test. Upper case letters compare data in rows and lower letters in columns.

Table 5 - pH and available P in soil after the two harvests of yam bean in a soil with low available P.

P-sources	pH (H_2O)	Available P (Mehlich)
	----------- mg kg^{-1} -----------	
Control with no P (P_0)	5.7 ab	5.4 d
Natural phosphate (NP)	6.2 a	16.8 c
Triple super phosphate	5.2 bc	31.0 b
Biofertilizer $_{20}$ (NP+S)	4.9 cd	6.2 d
Biofertilizer $_{5}$ (NP+S*)	4.8 cd	3.8 d
Biofertilizer $_{10}$ (NP+S*)	4.8 cd	21.1 c
Biofertilizer $_{15}$ (NP+S*)	4.6 cd	119.6 a
Biofertilizer $_{20}$ (NP+S*)	4.4 d	109.1 a
C.V. (%)	6.56	17.85

[1](NP+S*) = natural phosphate plus sulphur inoculated with *Acidithiobacillus*. (P+S) = natural phosphate plus sulphur without *Acidithiobacillus*.
[2]Values followed by different letters are different (P = 0.05), using the Tukey test.

Cruz et al. (1997) using different sources of nitrogen and phosphorus fertilizers on yam bean grown in a low available P soil. These authors suggested that for yam bean inoculated with specific and efficient rhizobia strains, it is not necessary nitrogen application for satisfactory yields.

The use of natural phosphates and liming is not an adequate and practical technology for heavy acid soils (Vasconcelos et al., 1986). In limed soils, Ernani et al. (2001) did not observe difference using various methods of P fertilizer application, and the best yields were obtained with soluble sources of phosphorus, in comparison to the natural phosphate.

Application of P caused a marked increase in total N and total P in plant shoots, and best results were obtained when biofertilizers with natural phosphate with sulphur and *Acidithiobacillus* and triple super phosphate were used. The positive impact of the biofertilizers produced with natural phosphate plus sulphur inoculated with *Acidithiobacillus* on total N and total P accumulated in shoots of yam bean holds great promise for improving input from these products as an alternative for partial or total substitution of soluble fertilizers.

Lombardi (1981) observed effect of “Alvorada” natural phosphate applied with sulphur and *Acidithiobacillus* on P total and growth of a tropical grass. Native bacteria in soil promoted sulphur oxidation as effective as the inoculated bacteria. However, the coefficient of variation obtained in the experiment was so high that was not possible to evaluate the positive effect of the sulphur inoculation with *Acidithiobacillus* when compared with the soil bacteria.

In the first harvest the native bacteria present in soil were not effective in the oxidation of sulphur applied in the biofertilizer without *Acidithiobacillus*, thus it seems that 90 days after the first harvest the soil bacteria may produce sulphuric acid and could act in P solubilization increasing available P in soil. Brasil & Muraoka (1997) observed a significant correlation between available P in soil and yield of plant biomass using Amazon soils, under natural phosphates and soluble fertilizers. Santos (2002), with the same soil used in this work, showed the positive effect of natural phosphate on available P in soil and growth of mimosa tree legume. The results are not so evident because of the use of natural phosphate pelleting the sulphur inoculated with *Acidithiobacillus*. Probably the reaction for sulphuric acid production was lowered due to the low input of air and water to react with the sulphur into the natural phosphate.

The soil pH, determined after the two consecutive harvests (180 days) was affected by the P treatments, especially when the biofertilizers produced with higher rates of sulphur and inoculated with *Acidithiobacillus* were applied but growth was not affected by the reduction in soil pH. The results are in accordance with data obtained by Ernani et al. (2001), with lime application in an acid soil with low available P. Probably the reduction in soil pH observed in the treatments with sulphur and *Acidithiobacillus* increased the available P and this parameter was considered by He et al. (1996) as a factor of greater effect in solubility of natural phosphates. Also Stamford et al. (2002, 2003), using sulphur inoculated with *Acidithiobacillus* in amendment of saline and sodic soils observed reduction in soil pH occurring until the total consumption of the added sulphur, promoting soil acidification varying from initial pH 8.2 up to pH 4.5 applying 1.8 t ha^{-1} of sulphur.

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